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Minimizing Yield Losses and Sanitary Risks through an Appropriate Combination of Fungicide Seed and Foliar Treatments on Wheat in Different Production Situations

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Abstract: Among the fungal diseases that affect wheat in temperate growing areas, *Septoria* Leaf Blotch (SLB) and *Fusarium* head blight (FHB) result in yield and sanitary risk losses that could be minimized through appropriate fungicide applications. Furthermore, the request from policy makers and the food market to reduce the use of chemical pesticides in agriculture has driven research in the direction of performant defense strategies with a reduced spraying of pesticides. The aim of this study was to evaluate the effects of different fungicide programs on the control of SLB and FHB, as well as on the grain yield and deoxynivalenol (DON) contamination of common wheat. Field experiments were carried out in 2016 and 2017 in North Italy. Two seed treatments (conventional vs. systemic) and four combinations of foliar fungicide applications (untreated control, application at the end of stem elongation, at flowering, and a double treatment at stem elongation and flowering) have been compared, according to a full factorial design, under two agronomic conditions: plowing vs. minimum tillage. Foliar sprayings at the end of stem elongation were found to be more effective in controlling SLB, while a triazole application at flowering was found to be an essential practice to reduce the FHB and DON contents. The double foliar treatment led to significant benefits, albeit only in the production situations with the highest SLB severity (e.g., in the 2017 experiment, after ploughing and the use of a conventional seed treatment). The systemic seed dressing led to a higher and prolonged STB protection, with significant canopy greenness during ripening in all the production situations. In 2017, which suffered from high disease pressure, the seed treatment with systemic fungicide led to a significant increase in grain yield (+5%), compared to the conventional one. The combination of the systemic seed treatment and the triazole application at flowering guaranteed the highest control of both SLB and FHB, maximized grain yield, and minimized DON contamination. This study provides useful information that could be used to evaluate appropriate fungicide programs, based on a combination of seed and foliar treatments, for wheat yield and sanity in distinct SLB and FHB diseases pressure scenarios.

Keywords: *Triticum aestivum*; *Septoria* leaf blotch; *Fusarium* head blight; deoxynivalenol; seed treatment

1. Introduction

In 2018, a total of 734 million tons of wheat was harvested across the globe, making it the third-largest grain crop in the world [1]. Of this, 33% was produced in Europe, where wheat, which is mainly cultivated as a winter crop, is the cereal that is grown the most, in terms of surface, and is a staple food for its citizens.

Among the various factors that could contribute significantly to reducing wheat yield, several diseases, such as root and foot rot complex, powdery mildew, rusts, *Septoria* leaf blotch complex (SLB), and *Fusarium* head blight (FHB), could have a negative

impact in temperate growing areas. It has been estimated that about 20% of the global wheat production is lost due to diseases every year [2,3]. Furthermore, the percentage of yield that could be lost, without plant protection, could exceed 70% in intensive temperate growing areas [4].

The seedlings, crowns, roots, and feet of wheat may be attacked by fungi (*Fusarium* spp., *Microdochium nivale*, *Bipolaris sorokiniana* and others), even in the early phenological stages, causing tissue discoloration, slow growth, a low tillering capacity, and reduced grain filling [5]. Foliar diseases are able to colonize the leaves, stems, and internodes of wheat, and have been associated with yield losses, due to a reduction in the photosynthetic life of the canopy. SLB, which is caused by the ascomycete *Mycosphaerella graminicola* (asexual stage *Zymoseptoria tritici*), is the main foliar disease of wheat in Europe [3]. Although leaves can be infected by SLB throughout the whole wheat life cycle, its effect on the loss of productivity and grain quality is more important when environmental conditions such as humidity and temperature are favorable for fungal growth during grain filling [6]. Crop protection strategies that are able to protect the flag leaf are required, since this leaf is responsible for 50% of grain filling assimilates [7]. The main agents of FHB in temperate areas, that is, *F. graminearum* and *F. culmorum*, are able to infect wheat spikelets at flowering, thereby causing total or partial premature senescence of the ears, in particular when rainy or wet periods occur between heading and the soft dough stage [8]. Both SLB and FHB are responsible for significant losses in yield and quality (low milling yield) whenever their attack strongly reduces grain test weight as a consequence of an early crop senescence [9]. In addition to grain yield loss, FHB is responsible for the accumulation of mycotoxins in the grains, and this remains a major hazard for human and animal health [10]. Deoxynivalenol (DON) is the most prevalent contaminant of wheat [11]. The European Commission (EC) has in fact set up regulatory limits to protect humans from exposure to this mycotoxin through cereal consumption (EC No. 1881/2006) [12].

The agronomic practices adopted for the prevention of fungal diseases mainly focus on minimizing the pathogen inocula using crop rotation [13] or soil tillage to incorporate previous crop debris [14], and the use of tolerant varieties [10,15]. However, in climatic conditions that are conducive to fungal diseases, the previously mentioned preventive measures might not be sufficient, and direct control, through the use of a fungicide application, is often necessary [16,17]. Applying a fungicide to seeds minimizes the risks associated with seedling mortality and allows a further control of the root and foot rot complex. Phenylpyrroles (e.g., fludioxonil) and triazoles (14 α -demethylation inhibitors, e.g., difeconazole, tebuconazole and prothioconazole) are the most widespread wheat seed dressing for this purpose [18]. On the other hand, spray applications to the canopy are necessary to control foliar disease and FHB. Fungicides containing triazoles, in particular metconazole and prothioconazole, applied at wheat flowering (growth stage, GS61, according to Zadoks [19]) are the most active molecules for the control of FHB infection and the consequent DON contamination [20]. This application timing also has a clear effect on delaying the decline of the green leaf area during grain filling and contributes to increasing grain yield [21]. Furthermore, in order to ensure a better control of SLB and other foliar diseases, fungicide spraying at a GS from the end of stem elongation (GS39) to booting (GS45) could guarantee a higher protection of the wheat canopy [22]. Such an application is in particular aimed at preserving the stay green of the flag leaf that has recently unrolled [23]. Strobilurin (chemical quinone outside inhibitors, QoIs) and carboxamide (succinate dehydrogenase inhibitors, SDHI) fungicides are generally used to obtain a high efficacy against the main foliar diseases and a marked physiological activity on plants, as they are able to induce a longer duration of the green flag leaf area than triazoles [24,25]. A double fungicide application of the fungicide at GS39 and GS61 is a crop protection strategy frequently adopted by farmers in temperate environments and where the agronomic conditions are more prone to fungal disease development, in order to maximize wheat yield [26].

A recent innovation on the market is the availability of a fungicide seed treatment characterized by a marked systemic activity, which is able to prolong the control of foliar disease, even in later growth stages. Among the systemic active ingredients (AI) that may be applied as a seed dressing, fluxapyroxad, a carboxamide fungicide, has proved to provide an effective and long-term disease control, through a foliar application, but also physiological benefits connected to an increase in leaf greening, delayed senescence, reduced cell damage, reduced stomatal conductance, an improved photosynthetic rate, and water use efficiency with a positive effect on grain yield [27].

The possibility of guaranteeing a profitable protection from the fungal diseases of winter wheat through the application of a systemic seed fungicide needs to be carefully evaluated, in order to check the role of these practices on the overall wheat protection programs and the interaction of such a fungicide with other fungicide treatments administered in spring. Considering the increasing request of lower pesticide applications in farming systems, as requested by politicians, supply chains and more in general by consumers, the possible substitution of a fungicide spray application with a seed dressing treatment would permit a clearly lower rate of active ingredients to be obtained per hectare.

The aim of the study was to evaluate the role of applying a systemic fungicide to wheat seeds in order to control fungal diseases and enhance grain yield and quality, considering the possibility of introducing this innovation into different crop protection programs for several agronomical and environmental conditions.

2. Materials and Methods

2.1. Experimental Site and Treatments

Field experiments were carried out in the 2015–2016 and 2016–2017 growing seasons in Buriasco (TO), in North-West Italy (44°54' N, 7°24' E; altitude 262 m.), in a sandy medium textured soil, classified as Typic Udifluvents (USDA classification), under naturally infected field conditions. Two adjacent experimental fields of winter wheat, one with a high agronomic risk of fungal diseases (related to the presence of previous crop residues on the soil) and the other with a low risk, were prepared each year. In both growing seasons, the previous crop was maize, grown according to a crop sequence normally applied in the growing area. The compared agronomic conditions were related to the tillage method, in order to favor diverse disease pressures:

- minimum tillage with double disk harrowing (15 cm depth), with previous maize crop residues left on the soil surface;
- fall ploughing (30 cm depth), which incorporated the maize debris into the soil, followed by disk harrowing to prepare a proper seedbed.

Different fungicides treatments were compared, under both agronomic conditions, according to a factorial combination of:

- a fungicide application as a seed dressing:
 - conventional: AI fludioxonil (Celest®, Syngenta Crop Protection S.p.A., Basel, Switzerland, fludioxonil 2.4%, 200 mL per 100 seed kg dose);
 - systemic: AI fluxapyroxad (Systiva®, BASF Agricultural Solutions S.p.A., Ludwigshafen, Germany, fluxapyroxad 28.7%, 150 mL per 100 seed kg dose).
- A foliar fungicide application:
 - an untreated control without any crop protection foliar treatment;
 - GS39, a single treatment at the end of stem elongation, in which a mixture of a strobilurin and a carboxamide (Priaxor®, BASF Agricultural Solutions, pyraclostrobin 150 g ha⁻¹ and fluxapyroxad 75 g ha⁻¹) was applied;
 - GS61, a single treatment at the beginning of flowering in which a triazole AI mixture (Osiris®, BASF Agricultural Solutions, epoxiconazole 75 g ha⁻¹ and metconazole 55 g ha⁻¹) was applied;

- GS39 + GS61, a double treatment through the combination of the previously reported single foliar applications.

The fungicide treatments were assigned to experimental units using a completely randomized block design, with four replicates. The plot size was 12 m² (6 m × 2 m). The normal agronomic techniques adopted in the growing area were applied. Briefly, the wheat cultivar used in both growing seasons was Aubusson, which has a medium susceptibility to FHB and SLB diseases (Limagrain Italia S.p.A., Busseto, PR, Italy). Planting was conducted in 12 cm wide rows on October 23, 2015, and October 20, 2016, at a seeding rate of 450 seeds m⁻². The experimental field received 140 kg N ha⁻¹ as a granular ammonium nitrate fertilizer (26% N), split between wheat tillering, GS 31, (60 kg N ha⁻¹) and the end of stem elongation, GS 39, (80 kg N ha⁻¹). At the end of tillering, a chemical weed control was carried out with Pinoxaden 3.03% + Clodinafop-propargyl 3.03% + Florasulam 0.76% + Cloquintocet-mexyl 0.76% (Traxos One®, Syngenta Crop Protection S.p.A.). The fungicides were applied at the manufacturers' recommended field rates, by means of a four-nozzle precision sprayer (Honda Agricultural Sprayer T-Jeet A110/04; Honda Motor Europe, Ltd., London, UK), using a fine mist at a slow walk to ensure an effective coverage. The delivery pressure at the nozzle was 300 kPa. In 2016, the fungicide treatments were conducted on 29 April at GS 39 and on 17 May at GS 61, while in 2017, they were conducted on 27 April and 17 May at GS 39 and GS 61, respectively.

2.2. Crop Assessments

2.2.1. Vegetation Index

A hand-held optical sensing device, GreenSeeker™ (Trimble®, Sunnyvale, CA, USA), was used to measure the normalized difference vegetation index (NDVI) from the first leaf stage (GS11) to the end of the grain-filling stage (GS85), in all plots.

The instrument was held approximately 60 cm above each single wheat plot, and its effective spatial resolution was 2 m × the full length of the plot (6 m). This assessment was performed every 2 weeks, until GS 39, and then every 7 days. The Area Under the Canopy Greenness Curve (AUCGC) was calculated, starting from the NDVI measurements, using the following formula:

$$AUCGC = \sum_i^{n-1} \{[(R_i + R_{i+1})/2] (t_{i+1} - t_i)\} \quad (1)$$

where R is the NDVI value, t is the time of observation, and n is the number of observations (12).

2.2.2. Septoria Leaf Blotch (SLB) Symptoms

The SLB severity was evaluated on the leaves at the beginning of flowering (GS61) and at the early dough stage (GS83) in each plot. Leaf disease was classified into six classes (0 = 0%; 1 = 2%; 2 = 5%; 3 = 10%; 4 = 25%; 5 = 50%; 6 ≥ 50%), according to visible symptoms [28]. At GS 61, the measurement was carried out on 75 leaves per plot (the last 5 leaves for 15 randomly selected plants). Instead, 15 randomly selected flag leaves and 15 penultimate leaves were used at GS 83. In 2016, the assessments were performed on May 16 (GS 61) and on June 15 (GS 83); they were instead carried out on May 17 and on June 7 in 2017.

2.2.3. Fusarium Head Blight (FHB) Symptoms

The incidence and severity of FHB was recorded in each plot by performing a visual evaluation of the disease on the grains at the early dough stage (GS83). The incidence was calculated as the percentage of ears with symptoms of the disease, using 200 randomly selected ears. The severity was calculated as the percentage of spikelets per ear with symptoms and was estimated on a scale from 0 to 7. Each numerical value corresponds to a percentage range of surfaces that exhibit visible symptoms of the disease [29], according

to the scheme: 1 = 0–5%; 2 = 6–15%; 3 = 16–30%; 4 = 31–50%; 5 = 51–75%; 6 = 76–90%; 7 = 91–100%. The assessment was recorded on June 15, in 2016, and on June 7, in 2017.

2.2.4. Grain Yield and Production Parameters

The plots were harvested, using a Walter Winterstaiger cereal plot combine harvester, on July 5, 2016, and July 13, 2017, and the grain yield results were adjusted to a 13% moisture content. Aliquots of 2 kgs of grain were taken from each plot to determine the test weight (TW), the thousands kernel weight (TKW), and the grain moisture content, using a GAC® 2000 Grain Analyzer (Dickens-John Auburn, IL, USA). TKW was determined on two 100-kernel sets for each sample (only whole seeds were considered) using an electronic balance. The harvested grains were mixed thoroughly, and an aliquot of 4 kg of grain was taken from each plot and ground completely using a Retsch ZM 200 (Retsch GmbH, Haan, Germany), fitted with a 1 mm aperture sieve. The resulting whole meal was analyzed for the DON content.

2.3. DON Analysis

The DON concentration was determined using the ELISA method, by means of direct competitive immunoassays RIDASCREEN® DON (R-Biopharm, Darmstadt, Germany), according to the method reported by Nguyen et al. [30].

2.4. Statistical Analysis

The normal distribution and homogeneity of variances were verified by performing the Kolmogorov–Smirnov normality test and the Levene test, respectively. The effect of the fungicide seed and foliar treatments on the AUCGC vegetation index, SLB incidence and severity, FHB incidence and severity, grain yield, TW, TKW, and DON content was tested by means of an analysis of variance (ANOVA), using a randomized complete block. ANOVA was used separately for each year and tillage, to explore the specific effects of the fungicide treatments under different environmental conditions. Multiple comparison tests were performed, according to the Ryan–Einot–Gabriel–Welsh F (REGW-F) method, on the treatment means ($p < 0.05$). Statistical analysis was performed with SPSS software, version 26 (IBM Corporation, Armonk, NY, USA, 2008).

3. Results

3.1. Meteorological Trends

The two growing seasons showed different meteorological trends throughout the wheat crop cycle (Table 1). The precipitations in the 2016–2017 growing season were 200 mm higher than in the 2015–2016 season, with the difference in rainfall mainly being concentrated during the leaf emission stages (November and December). The growing degree days (GDDs) were higher (+86 °C-day) from April to June in 2016–2017 than in 2015–2016.

Table 1. Monthly cumulative rainfall, rainy days, and growing degree days (GDDs) ¹ measured in the experimental areas from sowing (November) to harvesting (June) in the 2015–2017 period.

Month	Rainfall (mm)		Rainy Days (n°)		GDDs (Σ °C-Day) ²	
	2015–2016	2016–2017	2015–2016	2016–2017	2015–2016	2016–2017
November	2	257	4	7	293	250
December	1	77	0	5	188	159
January	5	12	4	3	151	111
February	164	62	12	13	188	175
March	90	69	7	8	295	356
April	96	51	9	6	430	412
May	117	77	11	11	516	558

June	34	103	14	7	636	698
November–March	261	477	27	36	1115	1050
April–June	246	231	34	24	1582	1668

¹ Data obtained from the Regione Piemonte agrometeorological service. ² Accumulated growing degree days for each experiment using a 0 °C base value.

3.2. SLB Symptoms and Vegetative Index

In both growing seasons, SLB affected the wheat canopy, although no symptoms of root rot or other foliar diseases were detected. The SLB incidence and severity in both GS61 and GS83 were higher in the 2016–2017 period than in the 2015–2016 growing season (Table 2). All the plant leaves showed SLB symptoms at GS83 (SLB incidence = 100%, data not shown). Furthermore, the SLB symptoms were clearly influenced by soil tillage, and in particular at this GS: the growth of the wheat under ploughing conditions resulted in a higher disease severity than under minimum tillage. At GS61, the systemic fungicide always significantly reduced SLB severity, by 47%, compared to the conventional seed treatment, except for the 2016 experiment under minimum tillage conditions. At GS83, the benefits, in terms of disease control of the systemic seed dressing, were significant for all the conditions and resulted in reductions of between 19% (2016, minimum tillage) and 27% (2017, ploughing). The fungicide application at GS39 significantly reduced SLB severity (–45%) for all the environmental conditions detected at flowering, compared to the untreated control. At the early dough stage, fungicide spraying at GS39 only resulted in a lower disease severity in the 2017 experiments. Compared to the untreated control, the disease symptoms during ripening were significantly lower than for the fungicide application at GS61 (–35%), while only under the ploughing conditions was a further reduction of SLB severity obtained with double spraying (GS39 + GS61).

Table 2. Effect of the fungicide seed and foliar treatments on *Septoria* Leaf Blotch (SLB) incidence and severity at flowering (GS 61), at early dough (GS83) and on the Area Under the Canopy Greenness Curve (AUCGC) detected during the vegetative stages, from the beginning of flowering (GS61) to the soft dough stage (GS85). 2015–2016 and 2016–2017 growing seasons, North-Italy.

Year	Soil Tillage	Factor	Source of Variation	SLB Incidence	SLB Severity	SLB Severity	AUCGC
				(GS61)	(GS61)	(GS83)	(GS61-GS85)
				%	%	%	NDVI-Day
2016	Minimum tillage	Seed treatment ¹	Conventional	25.6 a	2.0 a	26.1 a	27.3 b
			Systemic	21.0 a	1.5 a	21.2 b	29.0 a
			<i>p</i> -value ³	0.111	0.129	0.023	<0.001
		Foliar treatment ²	Untreated	31.3 a	2.6 a	29.3 a	27.1 b
			GS39	16.3 b	0.7 b	24.5 ab	28.7 a
			GS61	28.1 a	2.6 a	20.6 b	28.5 a
			GS39 + GS61	18.8 b	0.9 b	20.3 b	28.4 a
			<i>p</i> -value	<0.001	<0.001	0.012	0.023
		Seed × Foliar	<i>p</i> -value	0.457	0.349	0.228	0.816
2016	Ploughing	Seed treatment	Conventional	40.1 a	3.2 a	38.1 a	26.7 b
			Systemic	31.5 b	1.0 b	30.8 b	28.6 a
			<i>p</i> -value	0.009	<0.001	0.006	0.001
		Foliar treatment	Untreated	37.7 a	2.6 ab	42.8 a	26.4 b
			GS39	32.7 a	1.5 b	35.7 ab	28.1 a
			GS61	36.4 a	3.0 a	32.6 bc	27.9 ab
			GS39 + GS61	35.3 a	1.3 b	26.8 c	28.3 a
			<i>p</i> -value	0.650	0.043	0.001	0.024
		Seed × Foliar	<i>p</i> -value	0.446	0.268	0.042	0.268

2017	Minimum tillage	Seed treatment	Conventional	39.3 a	14.2 a	17.6 a	30.6 a
			Systemic	17.8 b	3.2 b	12.8 b	31.1 a
			<i>p</i> -value	<0.001	<0.001	0.012	0.063
		Foliar treatment	Untreated	32.7 a	10.9 a	27.9 a	29.9 b
			GS39	24.8 b	6.3 b	11.9 b	31.2 a
			GS61	34.8 a	11.3 a	13.2 b	30.9 a
			GS39 + GS61	21.9 b	6.3 b	8.0 b	31.6 a
			<i>p</i> -value	0.001	<0.001	<0.001	0.002
		Seed × Foliar	<i>p</i> -value	0.081	0.002	0.046	0.140
2017	Ploughing	Seed treatment	Conventional	56.1 a	22.2 a	31.9 a	29.4 b
			Systemic	44.1 b	11.7 b	23.3 b	30.2 a
			<i>p</i> -value	<0.001	<0.001	<0.001	0.005
		Foliar treatment	Untreated	55.6 a	19.9 a	50.3 a	27.9 c
			GS39	46.5 b	15.1 b	18.0 c	30.7 a
			GS61	53.8 a	18.6 a	32.4 b	29.5 b
			GS39 + GS61	45.8 b	15.0 b	9.9 d	31.0 a
			<i>p</i> -value	0.005	<0.001	<0.001	<0.001
		Seed × Foliar	<i>p</i> -value	0.796	0.100	<0.001	0.007

¹ Fungicide seed treatments: conventional (fludioxonil AI) and systemic (fluxapyroxad AI). ² Fungicide foliar treatment: untreated control; GS39, a single treatment at the end of stem elongation (pyraclostrobin + fluxapyroxad AI); GS61, a single treatment at the beginning of flowering (epoxiconazole + metconazole AI); GS39 + GS61, a double treatment through the application of a combination of GS39 and GS61. ³ Means followed by different letters are significantly different (the level of significance of the *p*-value is reported in the table), according to the REGW-F test.

The interaction between seed and foliar treatment was significant for SLB severity at GS83 in 2016 (ploughing) and in 2017 (under both ploughing and minimum tillage conditions). In all these production situations, the systemic seed dressing, without any further foliar applications, was able to significantly reduce SLB severity, reaching the same degree of protection obtained for the combination of conventional seed dressing and fungicide application at GS61 (Figure 1). When double foliar spraying was applied, no difference was recorded between the conventional and systemic seed treatments in any of the trials. In the production situation with the highest SLB pressure (2017, ploughing), the crop protection strategy with a single fungicide spraying was different according to the seed dressing. With the use of a systemic AI, the foliar applications at GS39 or GS61 resulted in a similar disease control, while with the conventional seed treatments, fungicide spraying at wheat flowering resulted in a significantly higher SLB severity.

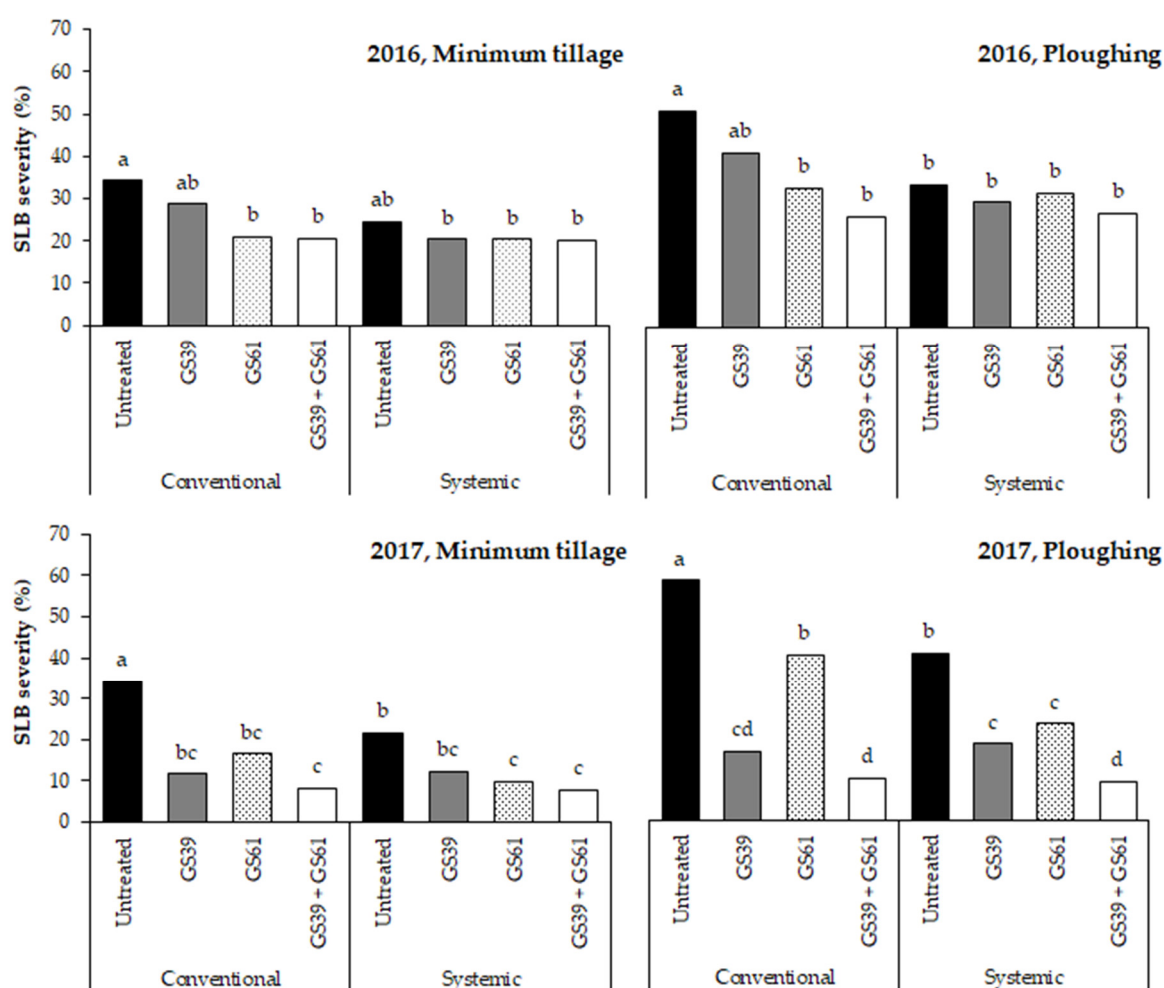


Figure 1. Effect of the fungicide seed ¹ and foliar ² treatments on *Septoria* Leaf Blotch (SLB) in different soil tillage and growing seasons (2015–2016 and 2016–2017) in North-Italy at the early dough stage (GS83). The bars in each experiment with different letters are significantly different (p -value < 0.05), according to the REGW-F test. The reported values are based on four replications. ¹ Fungicide seed treatments: conventional (fludioxonil AI) and systemic (fluxapyroxad AI). ² Fungicide foliar treatment: untreated control; GS39, a single treatment at the end of stem elongation (pyraclostrobin + fluxapyroxad AI); GS61, a single treatment at the beginning of flowering (epoxiconazole + metconazole AI); GS39 + GS61, a double treatment through a combination of the GS39 and GS61 applications.

The positive effect of seed and foliar treatments on SLB control was confirmed by the NDVI values detected during the growing season (Figures 2 and 3). Low values are related to a lower plant biomass and/or greenness status of the wheat canopy, and NDVI therefore reached the highest values from GS37 to GS69. The crop development was slightly slower under the minimum tillage conditions than under the ploughing conditions in both years. Only in 2016 did the systemic seed treatment result in lower NDVI values than the conventional one until GS23, with a slower emergence and development in the early stages. No difference was observed between the compared seed dressings from GS39 to GS69, while the systemic seed dressing resulted in a higher NDVI during grain filling than the conventional one, in all the production situations and considering the untreated control without foliar application, as a consequence of a delayed senescence (Figure 2). In both years, the seed treatment differences in NDVI were more visible under ploughing with higher SLB symptoms than under minimum tillage conditions.

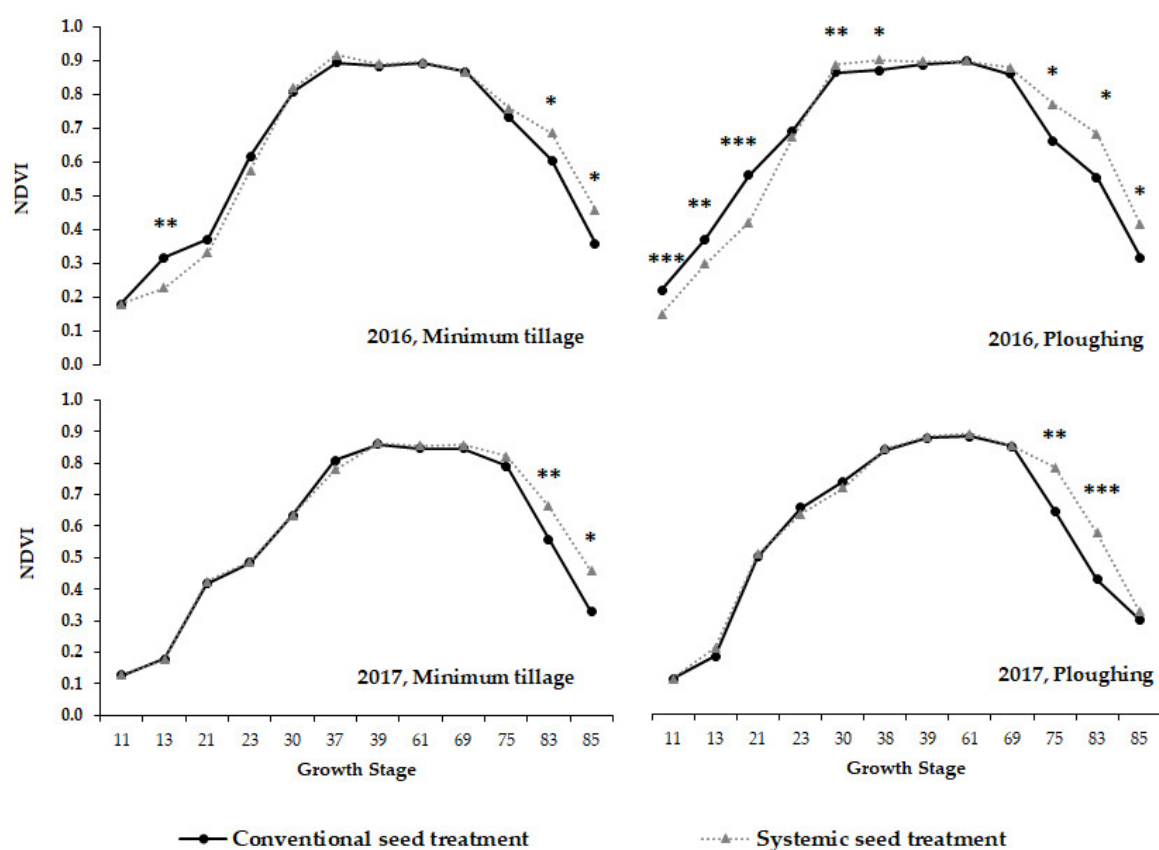


Figure 2. Effect of fungicide seed treatments ¹ on the normalized difference vegetation index (NDVI) measured from the first unfolded wheat leaf (GS11) to the soft dough stage (GS85) in different soil tillage and growing seasons (2015–2016 and 2016–2017) in North-Italy. ANOVA was performed for each NDVI value: * significant difference at the <0.05 level; ** significant difference at the 0.01 level; *** significant difference at the <0.001 level. The reported data are based on four replications of the untreated control, without any foliar fungicide. ¹ Fungicide seed treatments: conventional (fludioxonil AI) and systemic (fluxapyroxad AI).

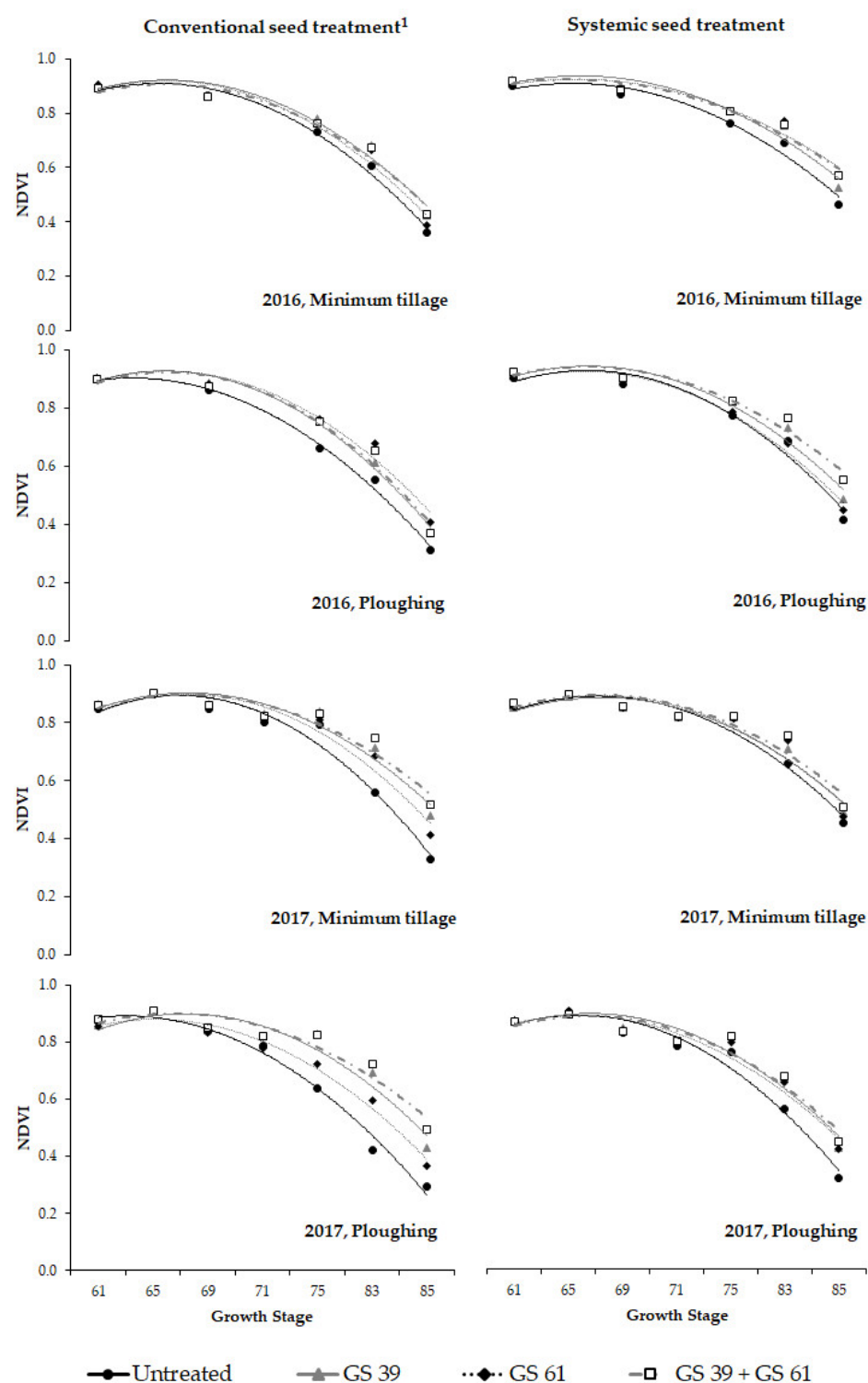


Figure 3. Effect of the fungicide foliar treatments ² on the normalized difference vegetation index (NDVI) measured from anthesis (GS61) until the soft dough stage (GS85), considering the seed treatments, soil tillage, and growing seasons (2015–2016 and 2016–2017). The reported values are based on four replications. ¹ Fungicide seed treatments: conventional (fludioxonil AI) and systemic (fluxapyroxad AI); ² Fungicide foliar treatment: untreated control; GS39, a single treatment at the end of stem elongation (pyraclostrobin + fluxapyroxad AI); GS61, a single treatment at the beginning of flowering (epoxiconazole + metconazole AI); GS39 + GS61, a double treatment through a combination of the GS39 and GS61 applications.

As far as the stay green evolution during grain filling is concerned (Figure 3), the application of the foliar fungicide led to higher NDVI values than the untreated control, with a more marked difference between the considered protection programs in the conventional seed treatment from the trials carried out in 2016 with minimum tillage than that in 2017 after ploughing. The systemic fungicide seed dressing alone (without any further fungicide application) was able to prolong the stay green, compared to the untreated conventional one. Moreover, when the systemic AI was applied to the seed, the differences between the foliar fungicide programs were smaller than those observed for the conventional seed dressing. Overall, the AUCGC vegetation index of the systemic seed dressing was significantly higher, that is, by 5%, than the conventional one (Table 2). A significant effect of the foliar treatments on AUCGC was observed for all the production situations (Table 2). Furthermore, only in 2017, under the ploughing conditions, were the differences between the single and double foliar fungicide treatments significant.

In this experiment, the interaction between the seed and foliar treatments was significant: when a systemic fungicide was applied as a seed dressing, a single foliar application at GS39 was able to guarantee a higher stay green during wheat ripening, while a further benefit of the double foliar treatments was observed for the conventional seed treatment.

3.3. FHB Symptoms and DON Content

The FHB incidence and severity and DON content are reported in Table 3. According to the SLB severity, the disease pressure was higher in 2017 than in 2016, as a consequence of the meteorological conditions, which were more prone to fungal development. As expected, FHB infection was higher under the minimum tillage conditions than in the ploughed soil, and the DON content in the kernels increased by 139% and 454% in 2016 and 2017, respectively.

No significant difference was observed for FHB incidence and severity between the fungicide seed treatments. Furthermore, the DON content was significantly higher in 2016 (+33%) for the systemic seed dressing than for the conventional one.

The FHB incidence and severity, and DON contamination were affected significantly by the fungicide foliar treatments (p -values < 0.001). The triazole application at GS61 significantly reduced the FHB symptoms and DON content (on average by 65%) in all the production situations, compared to the untreated control. The application of strobilurin and carboxamides (GS39 or GS39 + 61) could have resulted in a significantly higher DON content than the untreated control (2017, ploughing) or the single application at GS61 (2016, minimum tillage), respectively. The interaction between seed and foliar treatments was never significant as far as the DON content is concerned.

3.4. Grain Yield and Production Parameters

The grain yield and production parameters were only affected significantly by the seed treatment in 2017 (Table 4). The systemic seed dressing increased the grain yield (+5%) and TKW (+5%) more than the conventional one under both soil tillage conditions.

The effect of the foliar treatment on the grain yield was significant ($p < 0.01$) in 2016, under the ploughing conditions, and in 2017 in both trials. Furthermore, the interaction between the seed and foliar treatments was significant in this production situation. A significant increase in grain yield and TKW was recorded in 2016, albeit only for the conventional seed dressing, compared to the untreated control (Figures 4 and 5). A significant and similar increase in grain yield (+29%) and TKW (+10%) was recorded for both seed treatments in 2017, under minimum tillage conditions, as a result of the application of triazoles at flowering (GS61 or GS39 + GS61). In the same year, but in the ploughed plots, the highest TKW were obtained for the fungicide application at GS39 or at GS61, when the wheat seeds were treated with the conventional or the systemic AI (Figure 5).

Table 3. Effect of the fungicide seed and foliar treatments on *Fusarium* Head Blight (FHB) incidence and severity at the early dough stage (GS83) and on deoxynivalenol (DON) content in the 2015–2016 and 2016–2017 growing seasons in North-Italy.

Year	Soil Tillage	Factor	Source of Variation	FHB Incidence	FHB Severity	DON
				(GS83) %	(GS83) %	µg kg ⁻¹
2016	Minimum tillage	Seed treatment ¹	Conventional	38.6 a	5.4 a	940 b
			Systemic	42.3 a	4.8 a	1126 a
			<i>p</i> -value ³	0.245	0.427	0.025
		Foliar treatment ²	Untreated	58.6 a	8.3 a	1245 a
			GS39	50.7 a	9.2 a	1457 a
			GS61	25.7 b	1.4 b	549 c
			GS39 + GS61	26.7 b	1.5 b	882 b
			<i>p</i> -value	<0.001	<0.001	<0.001
			Seed × Foliar	<i>p</i> -value	0.590	0.413
2016	Ploughing	Seed treatment	Conventional	26.7 a	1.6 a	342 b
			Systemic	32.3 a	1.8 a	501 a
			<i>p</i> -value	0.130	0.573	0.012
		Foliar treatment	Untreated	43.0 a	2.7 a	604 a
			GS39	39.2 a	2.5 a	645 a
			GS61	16.1 b	0.7 b	244 b
			GS39 + GS61	19.7 b	0.9 b	193 b
			<i>p</i> -value	<0.001	<0.001	<0.001
			Seed × Foliar	<i>p</i> -value	0.096	0.813
2017	Minimum tillage	Seed treatment	Conventional	51.2 a	19.3 a	3682 a
			Systemic	51.3 a	19.2 a	3966 a
			<i>p</i> -value	0.974	0.981	0.521
		Foliar treatment	Untreated	65.9 a	30.5 a	6001 a
			GS39	69.4 a	31.5 a	6593 a
			GS61	36.7 b	8.0 b	1457 b
			GS39 + GS61	33.1 b	6.9 b	1243 b
			<i>p</i> -value	<0.001	<0.001	<0.001
			Seed × Foliar	<i>p</i> -value	0.737	0.287
2017	Ploughing	Seed treatment	Conventional	30.1 a	5.9 a	530 a
			Systemic	30.0 a	5.8 a	839 a
			<i>p</i> -value	0.954	0.926	0.064
		Foliar treatment	Untreated	53.0 a	11.2 a	853 b
			GS39	41.2 b	10.1 a	1414 a
			GS61	13.0 c	1.3 b	276 c
			GS39 + GS61	13.1 c	0.8 b	275 c
			<i>p</i> -value	<0.001	<0.001	<0.001
			Seed × Foliar	<i>p</i> -value	0.058	0.209

¹ Fungicide seed treatments: conventional (fludioxonil AI) and systemic (fluxapyroxad AI). ² Fungicide foliar treatment: untreated control; GS39, a single treatment at the end of stem elongation (pyraclostrobin + fluxapyroxad AI); GS61, a single treatment at the beginning of flowering (epoxiconazole + metconazole AI); GS39 + GS61, a double treatment through a combination of the GS39 and GS61 applications. ³ Means followed by different letters are significantly different (the level of significance of the *p*-value is reported in the table), according to the REGW-F test.

Table 4. Effect of the fungicide seed and foliar treatments on the grain yield, test weight (TW) and thousand kernel weight (TKW) in the 2015–2016 and 2016–2017 growing seasons in North-Italy.

Year	Soil Tillage	Factor	Source of Variation	Grain Yield t ha ⁻¹	TW kg hl ⁻¹	TKW g
2016	Minimum tillage	Seed treatment ¹	Conventional	7.4 a	81.8 a	46.4 a
			Systemic	7.5 a	81.4 a	44.9 a
			<i>p</i> -value ³	0.572	0.085	0.057
		Foliar treatment ²	Untreated	7.3 a	81.3 a	45.3 a
			GS39	7.5 a	81.5 a	45.1 a
			GS61	7.5 a	81.8 a	46.2 a
			GS39 + GS61	7.5 a	81.8 a	45.7 a
			<i>p</i> -value	0.468	0.303	0.817
		Seed × Foliar	<i>p</i> -value	0.465	0.032	0.485
2016	Ploughing	Seed treatment	Conventional	8.2 a	81.3 a	46.6 a
			Systemic	8.2 a	81.0 a	46.3 a
			<i>p</i> -value	0.527	0.598	0.362
		Foliar treatment	Untreated	7.8 b	80.5 a	45.0 b
			GS39	8.4 a	81.3 a	47.0 a
			GS61	8.4 a	81.4 a	46.8 a
			GS39 + GS61	8.3 a	81.7 a	47.0 a
			<i>p</i> -value	0.002	0.185	0.011
		Seed × Foliar	<i>p</i> -value	0.381	0.278	0.270
2017	Minimum tillage	Seed treatment	Conventional	7.1 b	72.2 b	42.1 b
			Systemic	7.4 a	73.2 a	42.9 a
			<i>p</i> -value	0.019	0.001	0.003
		Foliar treatment	Untreated	6.3 b	70.9 b	40.2 b
			GS39	6.6 b	71.1 b	41.0 b
			GS61	8.0 a	74.3 a	44.3 a
			GS39 + GS61	8.2 a	74.4 a	44.4 a
			<i>p</i> -value	<0.001	<0.001	<0.001
		Seed × Foliar	<i>p</i> -value	0.878	0.605	0.001
2017	Ploughing	Seed treatment	Conventional	7.6 b	72.9 a	41.0 b
			Systemic	8.1 a	72.7 a	43.9 a
			<i>p</i> -value	<0.001	0.622	<0.001
		Foliar treatment	Untreated	6.8 c	71.7 b	37.6 c
			GS39	7.9 b	72.6 ab	44.0 b
			GS61	8.0 b	72.9 ab	43.2 b
			GS39 + GS61	8.6 a	73.8 a	45.0 a
			<i>p</i> -value	<0.001	0.008	<0.001
		Seed × Foliar	<i>p</i> -value	<0.001	0.003	<0.001

¹ Fungicide seed treatments: conventional (fludioxonil AI) and systemic (fluxapyroxad AI). ² Fungicide foliar treatment: untreated control; GS39, a single treatment at the end of stem elongation (pyraclostrobin + fluxapyroxad AI); GS61, a single treatment at the beginning of flowering (epoxiconazole + metconazole AI); GS39 + GS61, a double treatment through a combination of the GS39 and GS61 applications. ³ Means followed by different letters are significantly different (the level of significance of the *p*-value is reported in the table), according to the REGW-F test.

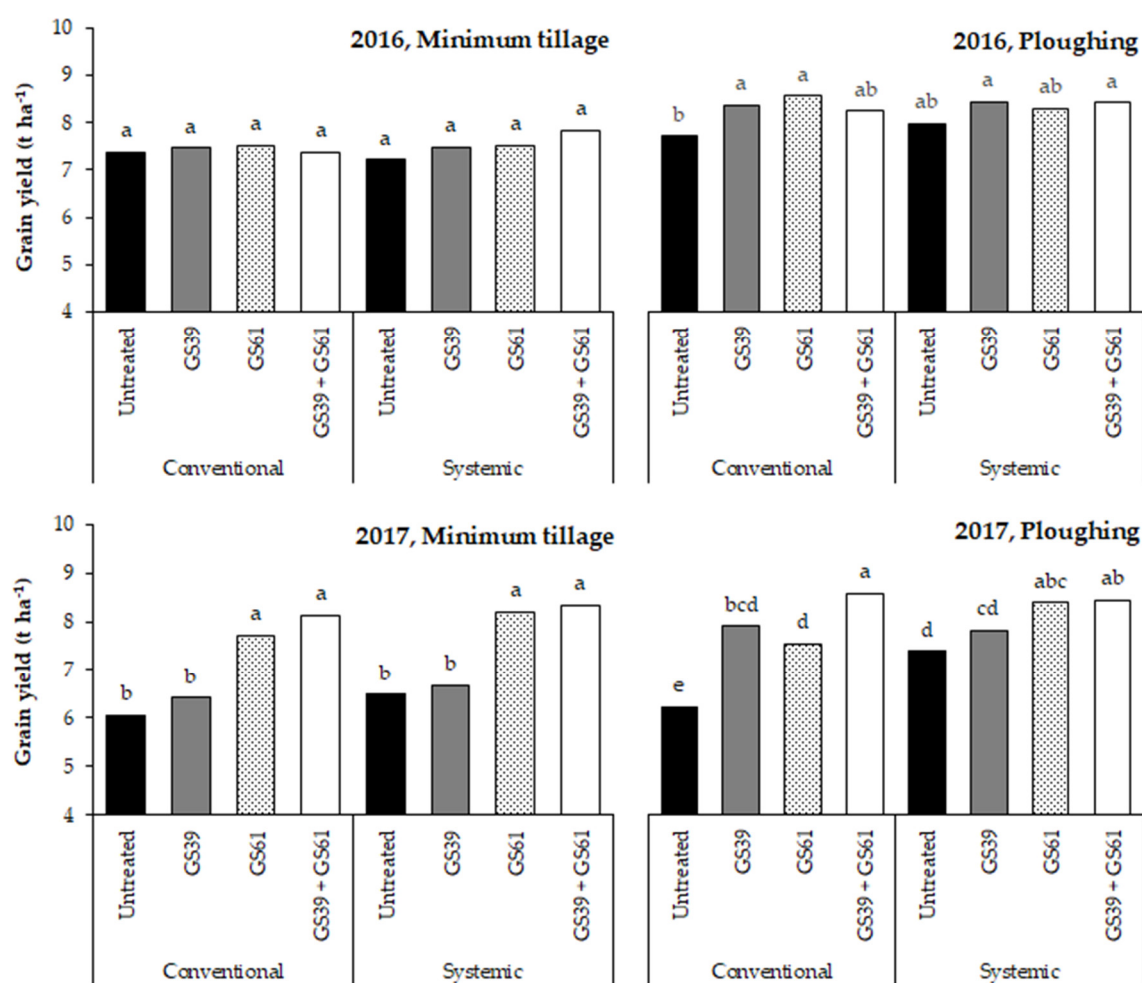


Figure 4. Effect of the fungicide seed ¹ and foliar ² treatments on the grain yield under different soil tillage conditions and in different growing seasons (2015–2016 and 2016–2017) in North-Italy. The bars in each experiment with different letters are significantly different (p -value < 0.05), according to the REGW-F test. The reported values are based on four replications. ¹ Fungicide seed treatments: conventional (fludioxonil AI) and systemic (fluxapyroxad AI); ² Fungicide foliar treatment: untreated control; GS39, a single treatment at the end of stem elongation (pyraclostrobin + fluxapyroxad AI); GS61, a single treatment at the beginning of flowering (epoxiconazole + metconazole AI); GS39 + GS61, a double treatment through a combination of the GS39 and GS61 applications.

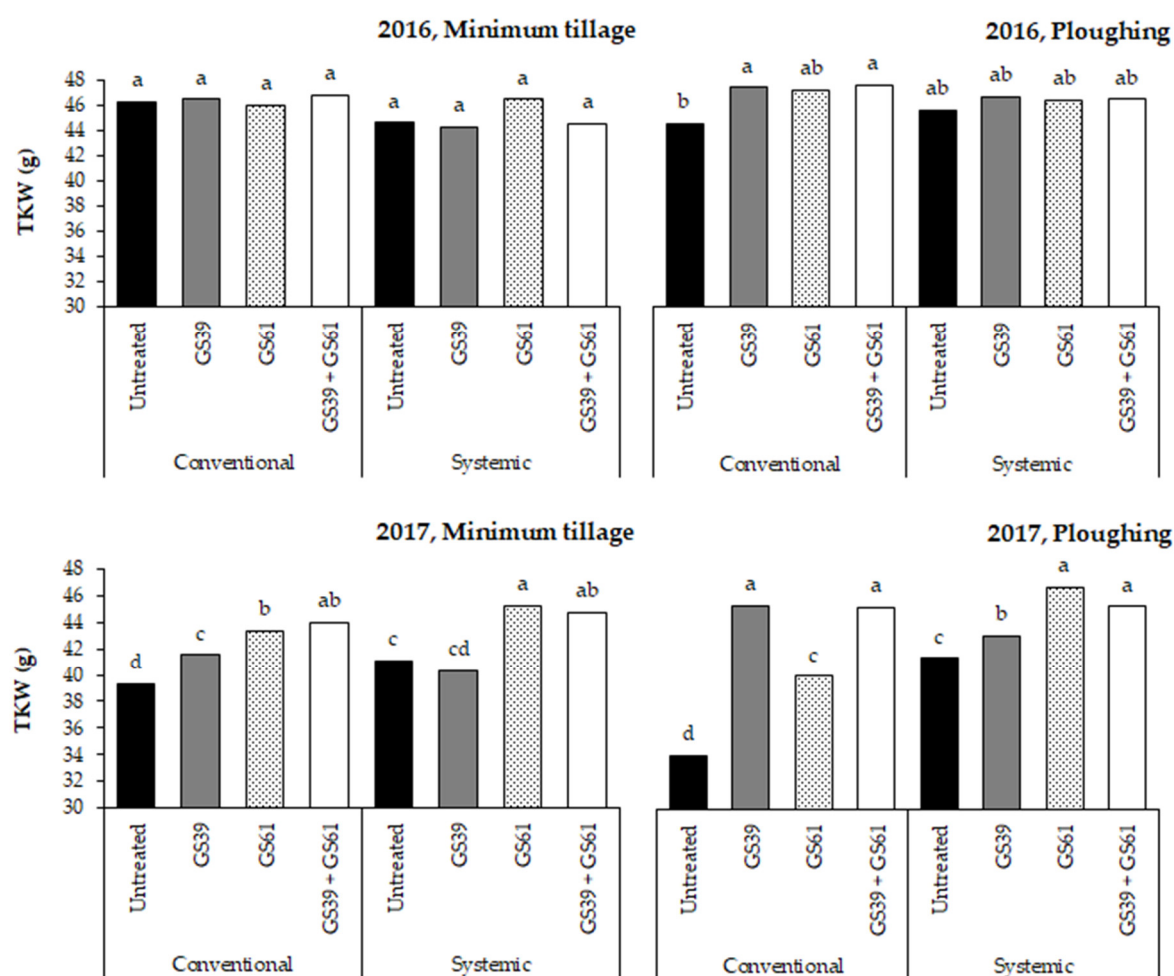


Figure 5. Effect of fungicide seed ¹ and foliar ² treatments on the thousand kernel weight (TKW) under different soil tillage conditions and in different growing seasons (2015–2016 and 2016–2017) in North Italy. The bars in each experiment with different letters are significantly different (p -value < 0.05), according to the REGW-F test. The reported values are based on 4 replications. ¹ Fungicide seed treatments: conventional (fludioxonil AI) and systemic (fluxapyroxad AI); ² Fungicide foliar treatment: untreated control; GS39, a single treatment at the end of stem elongation (pyraclostrobin + fluxapyroxad AI); GS61, a single treatment at the beginning of flowering (epoxiconazole + metconazole AI); GS39 + GS61, a double treatment through a combination of the GS39 and GS61 applications.

4. Discussion

The obtained results confirm the significant link between environmental conditions, agronomic practices, and fungal protection programs. The wetter and hotter spring months in 2017 led to more severe SLB and FHB infections and development than in 2016, thus showing larger differences between the compared fungicide strategies and a more effective role of both the seed and foliar treatments in preserving grain yield.

Furthermore, in both years, the presence of previous crop residues on the soil surface (minimum tillage) or their deep burial (ploughing) also clearly had an impact on the severity of the involved fungal species. It has been reported widely that the primary reservoir of FHB inoculum is debris from the previous crop, and DON contamination is more severe if the preceding crop is maize, since *Fusarium* survive longer on residues that do not degrade easily, and there is a direct relationship between debris biomass and fungal sporulation [31]. Thus, soil ploughing is the crop practice that is best able to reduce *Fusarium* infection on wheat [32]. On the other hand, under the considered conditions, the SLB severity on the wheat canopy was lower for the minimum tillage than for ploughing. In experiments carried out in Canada [33] and in Latvia [34], SLB was found to be more

frequent under conventional tillage, while tan spot (*Pyrenophora tritici-repentis*) was predominant under minimum tillage, thus suggesting a negative relationship between these pathogens. According to Bankina et al. [35], *Z. tritici* can survive in living plants as pycnidia, and the presence of plant debris on the soil surface could therefore be less important for the development of this disease. The marked difference in SLB symptoms observed in our study for different soil tillage operations and maize as the previous crop, would seem to suggest that the high level of *Fusarium* inoculum produced under minimum tillage conditions may have had a biocontrol effect, thereby reducing the infection of *Z. tritici*.

In all the production situations considered in the present study, the application of a foliar fungicide has led to a significant control of the fungal diseases, while the benefits, in term of grain yield have been observed more clearly for 2017, the year with the higher foliar and head disease pressure, than for 2016. Moreover, the collected data underline how the choice of the most appropriate fungal control strategies is closely related to the cropping systems. When the main target of a wheat crop protection program is FHB control, e.g., of the environments and cultivar, or crop practices, such as minimum tillage, which can lead to a higher risk of *Fusarium* infection and development, the application of a triazole fungicide at flowering should be mandatory to minimize the yield losses, to maintain acceptable TW values and to keep the contamination of DON below the regulatory limit thresholds. These results are in agreement with several research activities carried out in temperate growing areas, where applying triazoles at GS61 was found to be the best direct control solution against FHB infection and DON contamination [20,21,36]. Moreover, in previous studies, carried out in North Italy [10,37], this fungicide application led to a clear reduction, not only of DON, but also of several other mycotoxins and fungal metabolites produced by *F. graminearum* and *F. culmorum*, in addition to other emerging mycotoxins, such as enniatins and moniliformin, and metabolites produced by other fungal genus, such as *Alternaria* and *Claviceps*. As far as DON control efficacy is concerned, the double fungicide application (GS39 + GS61) did not result in any differences in most cases, compared to the single treatment (GS61), and the single application of the strobilurin and carboxamide mixture at the end of stem elongation did not lead to any advantages. Furthermore, the strobilurin and carboxamide mixture treatment carried out at GS39 could result in an increased risk of mycotoxin contamination, as a consequence of a slower dry down of the canopy during ripening, or a possible fungal competitive interaction phenomenon, with a shift of the fungal community. This change in the relative competition capacity among fungal species, as a result of the application of a control factor, which could result in an unexpected increase in the mycotoxin content, has been named the “flora inversion” phenomenon [10]. It has been widely reported that the application of strobilurin AI at wheat flowering is less effective against *F. graminearum* and *F. culmorum*, but it is able to significantly reduce the non-toxigenic *M. nivale*, and could therefore increase DON contamination [38,39]. In the present experiments, this possible effect on the fungal microbial shift was also observed for earlier applications than those at flowering.

Although the fungicide application at GS61 led to a clear reduction in SLB severity at the dough stages, and significantly prolonged the canopy stay green, the fungicide application at the end of stem elongation (GS39) in the production situations that made wheat more prone to SLB attacks (ploughing and conventional seed dressing in the 2017 experiment) led to the highest level of protection of the canopy, and in particular of the flag leaf, in the early ripening stages, thereby resulting in overall greater yield benefits. Similarly, the double foliar fungicide application led to a significant control of SLB at the dough stage and to an increase in yield, compared to the single fungicide treatment for cropping systems and environmental conditions highly prone to SLB. In the environments and genotype (durum wheat) with a high SLB pressure, the double treatment, with a strobilurin application at the stem elongation stage and an azole application at flowering, showed clear advantages, in terms of the delay of flag leaf senescence and yield, compared to the

treatment at flowering alone [40]. Several studies have reported a significantly higher capacity of strobilurin [21,41] and carboxamides [24,42] to control foliar disease and to maintain the green leaf area longer than triazoles, as well as of reducing the decline in flag leaf physiological activity and ensuring higher grain yields. In addition, both strobilurin and carboxamide have demonstrated the capacity to provide physiological benefits that further improve the photosynthetic rate of wheat [27,43–45] and other arable crops [46,47].

Whenever a conventional fungicide seed dressing is applied, the profitability of the double foliar treatment could increase, with an anticipation of the stem elongation timing (from GS32 to GS35), extending the interval of canopy protection and reducing early disease development. Moreover, the collected data highlight how the application of a seed dressing with a systemic carboxamide fungicide to winter wheat could change the overall foliar fungicide programs applied at spring. Compared to a conventional seed treatment, the use of fluxapyroxad AI, which is able to translocate inside the plant and to be active for longer, guarantees a greater and longer lasting protection, and also leads to significantly lower SLB severity at the dough stage. The protection activity of this solution led to a clear delay in canopy senescence, in particular during the ripening stage, as observed from the NDVI trend for the whole crop cycle. The overall higher AUCGC vegetation index is the result of the expression of a higher photosynthetic activity, which resulted in a significant increase in TKW and TW in the 2017 trials, and thus in grain yield [23]. As expected, the benefits of a systemic seed treatment were more effective in production situations in which the development of SLB is the target disease. Under these agronomical conditions, the prolonged activity of a seed dressing in controlling fungal disease throughout the vegetative stages cancels out the advantage of administering a specific treatment at the emission of the flag leaf (GS39), thereby leading to more effective benefits for the combination with a late application at flowering, a timing in which it is crucial to control FHB and mycotoxin contamination. Moreover, no further yield benefits have been observed in any of the trials with the double fungicide foliar application. Thus, the systemic fungicide seed treatments, with a prolonged fungal control, permit the need for foliar treatments to be reduced, thereby allowing the number of pesticide treatments and the overall AI quantity per surface unit applied to be reduced. Moreover, compared to spray applications, the use of seed dressing is an easy strategy to apply and is safer for farmers and non-target organisms [48].

Since *Fusarium* infections at flowering occur from the inoculum produced on the soil surface and from previous crop residues, which reach the ears mainly through dispersal in rain splashes [49], the seed treatment did not influence the FHB symptoms. Furthermore, in the year 2016, which showed a moderate FHB infection, the conventional phenylpyrrole seed dressing resulted in a significantly lower DON content than the systemic carboxamide one, which is less effective against *Fusarium* spp. Although the systemic growth of a *Fusarium* fungus originating from seeds is not able to reach the wheat heads, Moretti et al. [50] reported that a seed treatment prevented crown and root rot, and minimized the amount of DON that was able to translocate from the plant to the kernels because of its solubility in water. After comparing the role of seed treatments in different cropping systems, Blandino et al. [51] stated that a fludioxonil seed application on average reduced DON by 10% at harvesting, compared to an untreated control. Although the effect was not significant in 2017, the year with the highest level of FHB symptoms, the DON contamination was lower after the conventional seed dressing than after the systemic one. It has been hypothesized that the higher relative contribution of aerial head infection in that year, compared to the quantity of DON originating from the systemic infection, led to a less quantifiable effect of the seed dressing on mycotoxin contamination. Since the considered carboxamide fungicide is not able to efficiently prevent several of the fungal species that affect seedlings, crown and root rot, its combination with other systemic AI, such as triazoles, which are able to contribute to the control of foliar diseases [52], may represent a more efficient strategy for wheat seed dressing.

Among the other benefits of a fungicide seed dressing, but which was not quantified in the present study, the key role such a dressing plays in controlling soilborne and seed-borne pathogens that can attack seedlings and plants in the early growth stages should be mentioned, since no other effective direct control strategies can be applied [53]. Moreover, as previously reported, in addition to the protection endowed in the first growing stages, seed treatments with systemic and prolonged activity could permit a late shift of foliar application, thereby reducing the lack of control of diseases whenever the environmental conditions prevent an operator from entering a field to carry out foliar spraying. Rios et al. [54] highlighted that the early infection of leaves may have a negative impact on the physiology and photosynthesis of wheat.

In conclusion, our results, obtained under naturally infected field conditions, provide useful information to help evaluate the effects of different fungicide programs, based on the combination of seed and foliar treatments on wheat yield and sanity in distinct SLB and FHB disease pressure scenarios. The choice of the fungal control strategy is closely related to environmental (weather conditions, fungal population) and agronomic factors (mainly cultivar susceptibility, but also crop rotation and/or soil tillage as in the present study), thus it needs to be designed according to the overall fungal disease risk of the cropping system. In this context, the use of systemic seed treatments that are able to guarantee a prolonged protection from foliar diseases and to increase the duration of the green leaf area until the ripening stages, is a strategic practice that could be adopted to set up an effective crop protection program, in order to allow a greater sustainability of wheat cultivation to be obtained. Thus, because of the smaller amount of AI applied per hectare and the low risk for farmers and non-target organisms, seed application could represent a promising solution to reach the ambitious targets of a reduction in pesticide use and risks within the *Farm to Fork Strategy* proposed by the EU commission [55].

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